Attorney Docket No. 70058USPCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:	GROUP ART UNIT:
Michael G. Willits	1638
APPLICATION NO:	CONFIRMATION NO:
10/517,903	4683
FILED: December 10, 2004	EXAMINER: Keith O. Robinson

FOR: FLAVONOL EXPRESSING DOMESTICATED TOMATO AND METHOD OF PRODUCTION

Mail Stop Amendment
Commissioner for Patents
Box 1450
Alexandria, VA 22313-1450

Declaration of Michael Willits, Ph. D.
(Under 37 C.F.R. §1.132)

I, Michael Willits, declare as follows:

Background

1. I am an inventor of the invention disclosed and claimed in the above-referenced patent application.

2. I am a citizen of the USA. I am currently employed by Syngenta Biotechnology Inc. in Research Triangle Park, North Carolina, which as I understand is an affiliate of Syngenta Participations AG, the assignee of the referenced application. My present title is Scientist III. My curriculum vitae is attached to this Declaration as Exhibit A.

3. I have extensive experience in plant molecular biology. I received a Ph. D. in Molecular Biology from Stanford University, California, United States, in 1995. I received a Bachelor's Degree in Biochemistry from North Carolina State University, North Carolina, United States, in 1989. I was also educated in the areas of genetics and scientific research.

4. I have written numerous publications concerning nitrogen fixation in alfalfa and plant molecular biology thereof for many journals, including publications in The Plant Cell, Molecular Plant-Microbe Interactions, the Journal of Bacteriology, Phytochemistry, Plant Cell Reports, and the Journal of Agricultural and Food Chemistry. A list of my publications is provided at the end of the curriculum vitae.

5. I have performed, coordinated and/or supervised the experiments described below.

Written Description

6. I have reviewed portions of the Office Action dated July 16, 2007 (hereafter "OA-7/16/07", attached hereto as Exhibit B), in which the Examiner rejected the pending claims as allegedly failing to comply with the written description requirement and as anticipated by both Goffedra et al (Theor. Appl. Genet. 78:210-216, 1989) and Stewart et al (J. Agric. Food Chem. 48: 2663-2669, 2000). I have also reviewed the Goffedra reference, attached hereto as Exhibit C.

7. Based on my understanding, the above-referenced patent application is being rejected because we claim an *L. esculentum* plant having the desired flavonol characteristics; however, in the application, we do not show "possession" of a plant of this type. We describe the *L. pennellii* plant with those characteristics, and then the *L. esculentum* X *L. pennellii* hybrid, but in lieu of describing how we obtained an *L. esculentum* plant with the desired characteristics, we say that traditional tomato breeding techniques are used to obtain this plant from the F1 hybrids.

8. It is also my understanding that the statement we made in a publication (J. Agric. Food Chem. 2005, 53:1231-1236) may be leading to the idea that an *L. esculentum* variety cannot be created from the F1 hybrids.

9. However, the statement being cited by the Examiner was not made to suggest that the breeding process to obtain an *L. esculentum* from the F1 hybrids with the desired flavonol characteristics was not possible. In actuality, the *L. esculentum* variety was not created because we came to the end of our project and we did not have funding or the facilities to start a breeding program.

10. We produced a hybrid which demonstrated the transfer of the trait to the hybrid tomato fruit (in this context, the term 'very difficult' used in the publication referred only to this trait and not to the overall breeding process). The creation of an *L. esculentum* variety maintaining the production of flavonoids in the fruit could be accomplished by any competent breeder within well known, standard "ordinary skill in the art" breeding methods.

11. A person of ordinary skill in the art would recognize upon reading of the specification that to obtain the claimed *L. esculentum* plant, they would only need to create a *L. esculentum X L. pennellii v. puberulum* hybrid, select a progeny with the desired flavonol trait, and then develop a breeding scheme involving standard backcrossing and self-pollination methods to obtain an *L. esculentum* plant with the desired characteristics. However, as stated above, we lacked the funding and facilities to undertake this breeding process.

Novelty

12. As for the rejection over the publication in Theor. Appl. Genet. 1989, 78:210-216, the Examiner argues "that any cross of these two species would inherently possess the flavonol characteristics of your invention." That is only true if you were aware of this trait and then set up a breeding program to specifically introgress this trait, which is actually determined by two different, unlinked genes.

13. Being aware of the trait is not a simple matter, because a simple scan of flavonoid production in wild species, including *L. pennellii*, does not identify lines that are suitable to confer the trait. We analyzed the expression of flavonoid pathway genes in the fruit and determined why *L. esculentum* does not make the appropriate flavonoids in the fruit. Then we found a wild line (in this case *L. pennellii*) that would correct the gene expression.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

10/16/2007
Date

Michael Willits
Michael Willits, Ph. D

EXHIBIT A

Michael Gregory Willits
Curriculum Vitae

804 Winter Hill Drive
Apex, North Carolina 27502

Phone: (919) 541-8635 (work)
(919) 303-4444 (home)

Education:

1995 Ph.D. in Molecular Biology from the Department of Biological Sciences at Stanford University

Characterized the sulfate activation genes of *Rhizobium meliloti* at the molecular and biochemical levels in Dr. Sharon Long's laboratory.

- Analyzed the expression patterns of two sulfate activation loci, *nodPQ*$_1$ and *nodPQ*$_2$, which are involved with Nod factor sulfation in *Rhizobium meliloti*.
- Cloned and characterized a third sulfate activation locus, *cysHDN*, involved with cysteine biosynthesis.
- Determined biochemically that *Rhizobium meliloti* utilizes an altered pathway for sulfate activation during cysteine biosynthesis that is more similar to plant than bacterial systems.

1989 B.S. in Biochemistry from the Department of Biochemistry at North Carolina State University

Professional Experience:

1/07-present Scientist III at Syngenta Biotechnology Inc.

Member of the HTC and Vector Pipeline team in CSCT. Technical Lead responsible for finding a mesotrione-degrading gene for the HPPD Soybean project. Head of the QC Submissions Funtional Team, responsible for coordinating activities concerning plant binary QC submissions and for liaising with the Genie Production Team to modify Genie to work with the Vector Pipeline processes. Head of the Science Stream in Syngenta Science Live, responsible for generating a Science Fair that highlighted SBI, Greensboro and Global Syngenta Science. Member of the SBI Tour Team.

1/06-12/06 Scientist III at Syngenta Biotechnology Inc.

Member of an ad hoc team formed to provide molecular data to Regulatory Sciences concerning the regulated transgenic event Bt10. In the absence of complete sequence data, it was necessary to provide a detailed map of the genomic region surrounding and including the Bt10 insert.

1/05-12/05 Scientist III at Syngenta Biotechnology Inc.

Member of a team working on broad spectrum insect resistance in maize. Participated in the directed mutagenesis of VIP3E in an effort to increase insecticidal activity and decrease phytotoxicity. Investigated sub-cellular localization of VIP3E in order to alleviate phytotoxicity.

4/02-12/05 Staff Scientist II at Syngenta Biotechnology Inc.

Completed work in the Health and Nutrition Group, including writing four papers and one patent. Transferred to the Fiber Processing Group and participated in identifying genes involved in corn fiber degradation and methods of expressing such genes in corn seed.

1/99-3/02 Staff Scientist I at Syngenta Biotechnology Inc.

Worked in the Natural Resistance Group in an effort to identify disease resistance genes. After transferring to the Health and Nutrition Group, engineered tomato plants to contain high levels of flavonoids.

11/95-12/98 Post-Doctoral Associate at Novartis Agribusiness Biotechnology Research, Inc.

Investigated the signal transduction pathway leading to the induction of systemic acquired resistance in tobacco and Arabidopsis in Dr. John Ryals' laboratory.

10/90-12/90 Teaching Assistant in Biochemistry and Genetics at Stanford University

1/90-3/90 Teaching Assistant in Cell Biology at Stanford University

5/89-8/89 Biologist at NIEHS, Research Triangle Park, North Carolina

Characterized several genes that were differentially expressed in the presence of TGF-β using Northern blots and DNA sequencing in Dr. Anton Jetten's laboratory.

5/88-10/88 Research Apprentice with R.J. Reynolds Co. at North Carolina State University

Studied two double-stranded mitochondrial RNA molecules associated with Cytoplasmic Male Sterility in maize in Dr. Paul Sisco's laboratory.

5/87-8/87 Lab Assistant with Dr. Paul Sisco at North Carolina State University

Mapped morphological traits on the long arm of maize chromosome one utilizing various DNA analysis techniques.

Abstracts and Publications:

Willits, M.G., Deluca, V., Graser, G., Kramer, C.M., Prata, R.T.N. 2005. Flavonol expressing domesticated tomato and method of production. Patent Number US 2005/0160495 A1.

Willits, M.G., Kramer, C.M., Prata, R.T.N., De Luca, V., Potter, B.G., Steffens, J.C. and Graser, G. 2005. Utilization of the genetic resources of wild species to create a nontransgenic high flavonoid tomato. Journal of Agricultural and Food Chemistry 53, 1231-1236.

Sigareva, M., Spivey, R., Willits, M.G., Kramer, C.M. and Chang, Y.-F. 2004. An efficient mannose selection protocol for tomato that has no adverse effect on the ploidy level of transgenic plants. Plant Cell Reports 23, 236-245.

Willits, M.G., Giovanni, M., Prata, R.T.N., Kramer, C.M., De Luca, V., Steffens, J.C. and Graser, G. 2004. Bio-fermentation of modified flavonoids: an example of in vivo diversification of secondary metabolites. Phytochemistry 65, 31-41.

Kramer, C.M., Prata, R.T.N., Willits, M.G., De Luca, V., Steffens, J.C. and Graser, G. 2003. Cloning and regiospecificity studies of two flavonoid glucosyltransferases from *Allium cepa*. Phytochemistry 64, 1069-1076.

Salmeron, J.M., Weislo, L.J. and Willits, M.G. 2003. Plant genes and uses thereof. United States Patent 6,528,702.

Keating, D.H., Willits, M.G. and Long S.R. 2002. A *Sinorhizobium meliloti* lipopolysaccharide mutant altered in cell surface sulfation. Journal of Bacteriology 184, 6681-6689.

Friedrich, L., Lawton, K., Dietrich, R., Willits, M., Cade, R. and Ryals, J. 2001. NIM1 overexpression in Arabidopsis potentiates plant disease resistance and results in enhanced effectiveness of fungicides. Molecular Plant-Microbe Interactions 14, 1114-1124.

Gupta, V., Willits, M.G. and Glazebrook, J. 2000. *Arabidopsis thaliana* EDS4 contributes to salicylic acid (SA)-dependent expression of defense reponses: evidence for inhibition of jasmonic acid signalling by SA. Molecular Plant-Microbe Interactions 13, 503-511.

Abola, A.P., Willits, M.G., Wang, R.C. and Long, S.R. 1999. Reduction of adenosine-5'-phosphosulfate instead of 3'-phosphoadenosine-5'-phosphosulfate in cysteine biosynthesis by *Rhizobium meliloti* and other members of the family Rhizobiaceae. Journal of Bacteriology 181, 5280-5287.

Willits, M.G. and Ryals, J.A. 1998. Determining the relationship between salicylic acid levels and systemic acquired resistance induction in tobacco. Molecular Plant-Microbe Interactions 11, 795-800.

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.-Y., and Hunt, M.D. 1996. Systemic acquired resistance. The Plant Cell 8, 1809-1819.

Willits, M.G. 1995. Molecular and biochemical characterization of sulfate activation genes in *Rhizobium meliloti*. Thesis. Stanford University, Stanford, California.

Willits, M.G. and Long, S.R. Expression and biochemical function of *NodPQ*$_1$ and *NodPQ*$_2$ in *Rhizobium meliloti*. Keystone meeting on Signal Transduction in Plants. (Hilton Head, SC, 1995).

Willits, M.G. and Long, S.R. Growth-rate dependent regulation of *NodPQ*$_1$ and *NodPQ*$_2$ in *Rhizobium meliloti*. Seventh International Symposium on Molecular Plant-Microbe Interactions. (Edinburgh, Scotland, June 1994).

EXHIBIT B

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/517,903	12/10/2004	Michael G Willits	70058USPCT	4683

22847 7590 07/16/2007

SYNGENTA BIOTECHNOLOGY, INC.
PATENT DEPARTMENT
3054 CORNWALLIS ROAD
P.O. BOX 12257
RESEARCH TRIANGLE PARK, NC 27709-2257

EXAMINER	
ROBINSON, KEITH O NEAL	
ART UNIT	**PAPER NUMBER**
1638	

MAIL DATE	DELIVERY MODE
07/16/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/517,903	WILLITS ET AL.	
	Examiner	Art Unit	
	Keith O. Robinson, Ph.D.	1638	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 May 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-36* is/are pending in the application.

4a) Of the above claim(s) *11-36* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-10* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *10 December 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *1/13/2006*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election without traverse of Group I (claims 1-10) in the reply filed on May 2, 2007 is acknowledged. The requirement is made FINAL.

2. Claims 11-36 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected group, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on May 2, 2007.

3. Claims 1-10 are under examination.

Claim Objections

4. Claims 8-10 are objected to because of the following informalities: Claims 8 and 9 should begin with the word "A" and claim 10, line 1 should read - - or a part thereof, - - because only a single invention can be claimed.

Appropriate correction is required.

Claim Rejections - 35 USC § 112, first paragraph – Written Description

5. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claims 1-10 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed,

had possession of the claimed invention. The claims read on any non-transgenic domesticated *L. esculentum* plant having flavonol content in the flesh of the fruit of said plant that is greater than 0.5 μg/mgwt.

See MPEP 2163(I) where it states "[t]o satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. See, e.g., Moba, B.V. v. Diamond Automation, Inc., 325 F.3d 1306, 1319, 66 USPQ2d 1429, 1438 (Fed. Cir. 2003); Vas-Cath, Inc. v. Mahurkar, 935 F.2d at 1563, 19 USPQ2d at 1116".

Based on the evidence disclosed in the specification, it appears that Applicant is not in possession of any non-transgenic domesticated *L. esculentum* plant having a flavonol content in the flesh of the fruit of said plant that is greater than 0.5 μg/mgwt. The specification states that "*L. pennellii v. puberulum* (LA1926) shows strong expression of all investigated flavonol biosynthetic genes in both the fruit peel and flesh...[and thus,]... *L. pennellii v. puberulum* (LA1926) was chosen as a crossing partner to introgress flavonol production into *L. esculentum*" (see page 10, lines 22-25); however, the specification does not show that Applicant was in possession of a *L. esculentum* plant having a flavonol content in the flesh of the fruit of said plant that is greater than 0.5 μg/mgwt.

Also see MPEP 2163.02 where it states, "[a]n objective standard for determining compliance with the written description requirement is, "does the description clearly

allow persons of ordinary skill in the art to recognize that he or she invented what is claimed." In re Gosteli, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989)".

In the instant invention, one of ordinary skill in the art would only recognize that Applicant has invented any non-transgenic domesticated *L. esculentum* plant having a flavonol content in the flesh of the fruit of said plant that is greater than 0.5 μg/mgwt.

MPEP 2163.02 further states, "[u]nder Vas-Cath, Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Fed. Cir.1991), to satisfy the written description requirement, an applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, and that the invention, in that context, is whatever is now claimed".

In the instant case, Applicant has not conveyed with reasonable clarity to those skilled in the art that, as of the filing date sought, Applicant was in possession of any non-transgenic domesticated *L. esculentum* plant having a flavonol content in the flesh of the fruit of said plant that is greater than 0.5 μg/mgwt.

The only non-transgenic *Lycopersicon* lines that showed flavonol content in the flesh of the fruit were LA1963, LA2884 and LA1926; however, none of these lines were *L. esculentum* plants. In addition, Applicant shows a *L. esculentum X L. pennellii v. puberulum* hybrid that possesses flavonol content in the flesh of the fruit, but it does not appear to be a non-transgenic domesticated *L. esculentum* plant (see page 14, Table 3), but Willits et al (J Agric Food Chem 53: 1231-1236, 2005) teach, "[i]t turned out to be difficult to produce tomatoes from these crosses, and the fruit was invariably seedless...it was not possible to further analyze progeny from the F1 hybrid...a

breeding program to remove interspecific breeding barriers between *L. pennellii* and *L. esculentum* is required in order to obtain fertile hybrids for subsequent analysis of the high flavonoid trait in the next generation" (see page 1235, 1st column, 1st full paragraph).

Therefore, it is unclear how Applicant was in possession of a non-transgenic domesticated *L. esculentum* plant having flavonol content in the flesh of the fruit of said plant that is greater than 0.5 μg/mgwt when the prior art teaches that it was not possible to further analyze progeny from the F1 hybrid because of interspecific breeding barriers between *L. pennellii* and *L. esculentum* .

Thus, based on the disclosure of the specification, Applicant has not shown possession any non-transgenic domesticated *L. esculentum* plant having a flavonol content in the flesh of the fruit of said plant that is greater than 0.5 μg/mgwt.

Claim Rejections - 35 USC § 102

7. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

8. Claims 1-10 are rejected under 35 U.S.C. 102(b) as being anticipated by Goffreda et al (Theor Appl Genet 78: 210-216, 1989). The claims read on any non-transgenic domesticated *L. esculentum* plant having a flavonol content in the flesh of the fruit of said plant that is greater than 0.5 μg/mgwt.

The specification states, "the introduction of the genetic factors from *L. pennellii* into the hybrid plant results in the expression of the flavonol biosynthetic genes and subsequent production of flavonols and flavonol glucosides in the peel and also in the flesh" (see page 14, lines 11-14). Thus, the claims are interpreted as any tomato plant having genes introgressed from *L. pennellii*.

Goffreda et al disclose a *L. esculentum* plant having genes introgressed from *L. pennellii* (see page 212, Tables 1-3) and thus, would inherently possess a flavonol content in the flesh of the fruit of said plant that is greater than 0.5 μg/mgwt based on the disclosure of the specification as stated above.

See *In re Thorpe*, 227 USPQ 964, 966 (Fed. Cir. 1985), which teaches that a product-by-process claim may be properly rejectable over prior art teaching the same product produced by a different process, if the process of making the product fails to distinguish the two products. See *In re Best*, 195 USPQ 430, 433 (CCPA 1997), which teaches that where the prior art product seems to be identical to the claimed product, except that the prior art is silent as to a particularly claimed characteristic or property, then the burden shifts to Applicant to provide evidence that the prior art would neither anticipate nor render obvious the claimed invention.

9. Claims 1-10 are rejected under 35 U.S.C. 102(b) as being anticipated by Stewart et al (J Agric Food Chem 48: 2663-2669, 2000). The claims read on any non-transgenic domesticated *L. esculentum* plant having a flavonol content in the flesh of the fruit of said plant that is greater than 0.5 μg/mgwt.

Stewart et al disclose non-transgenic domesticated *L. esculentum* plants having a flavonol content in the flesh of the fruit of said plant that is greater than 0.5 μg/mgwt (see page 2667, Table 2).

See *In re Thorpe*, 227 USPQ 964, 966 (Fed. Cir. 1985), which teaches that a product-by-process claim may be properly rejectable over prior art teaching the same product produced by a different process, if the process of making the product fails to distinguish the two products. See *In re Best*, 195 USPQ 430, 433 (CCPA 1997), which teaches that where the prior art product seems to be identical to the claimed product, except that the prior art is silent as to a particularly claimed characteristic or property, then the burden shifts to Applicant to provide evidence that the prior art would neither anticipate nor render obvious the claimed invention.

Conclusion

10. No claims are allowed.

11. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Keith O. Robinson, Ph.D. whose telephone number is (571) 272-2918. The examiner can normally be reached Monday - Friday 7:30 a.m. - 4:30 p.m. EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anne Marie Grunberg can be reached at (571) 272-0975. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

12. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Keith O. Robinson, Ph.D.

July 5, 2007

MEDINA A. IBRAHIM
PRIMARY EXAMINER

EXHIBIT C

Theor Appl Genet (1989) 78: 210–216

Inheritance of potato aphid resistance in hybrids between *Lycopersicon esculentum* and *L. pennellii*

J. C. Goffreda and M. A. Mutschler

Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853, USA

Received September 8, 1988; Accepted March 29, 1989
Communicated by A. R. Hallauer

Summary. The potato aphid, *Macrosiphum euphorbiae* Thomas, is an important pest of tomato, *Lycopersicon esculentum* Mill., because it transmits tomato viruses and directly reduces crop yields by its feeding. This study was conducted to determine whether the wild tomato species, *Lycopersicon pennellii* (Corr.) D'Arcy, would be useful as a source of potato aphid resistance for tomato. Type IV trichome density and aphid resistance were assessed in six generations (P_1, P_2, F_1, F_2, BC_1P_1, and BC_1P_2) from crosses between *L. pennellii* (LA 716) and two tomato cultivars, New Yorker and VF Vendor. Weighted least-squares were used in joint scaling tests to estimate the relative importance of gene effects on type IV trichome density and potato aphid resistance of the hybrids. A simple additive-dominance model adequately explained the variation in type IV trichome density. Models which included digenic epistatic effects were required to explain the variation in aphid resistance. Standard unit heritability estimates of aphid resistance in the backcross to *L. esculentum* were obtained by regression of BC_1F_2 offspring families on BC_1F_1 parents. Regression coefficients and heritability estimates varied between years with the level and uniformity of the aphid infestation. In the 1985–1986 growing seasons, when aphid infestations were uniform, aphid resistance exhibited a moderate level of heritability ($29.8\% \pm 14.1\%$ and $47.1\% \pm 11.5\%$ in New Yorker and VF Vendor backcross populations, respectively). The non-uniform aphid infestation of 1984 resulted in lower heritability estimates in the 1984–1985 growing seasons ($16.1\% \pm 15.7\%$ and $21.9\% \pm 14.8\%$ in the New Yorker and VF Vendor backcross populations, respectively). Selection for potato aphid resistance would probably be most efficient if it were delayed until gene combinations are fixed in later generations, because of the large epistatic effects and the low heritability of this trait in seasons with variable aphid infestations.

Key words: Generation means – Heritability – Insect resistance – Joint scaling tests – Tomato

Introduction

The potato aphid, *Macrosiphum euphorbiae* Thomas, is an important pest of tomato, *Lycopersicon esculentum* Mill., when environmental conditions are favorable for rapid aphid growth and reproduction (Quiros et al. 1977; Walker et al. 1984). The potato aphid is not only damaging to tomato when high aphid populations compete with the developing fruit for nutrients and carbohydrates; even low aphid infestations can indirectly cause significant reductions in yield, since this and other aphid species are efficient vectors of many tomato viruses (Lange and Bronson 1981; Kennedy et al. 1962). Currently, chemical insecticides are the only effective means of aphid control. However, there is evidence that some aphid species are developing resistance to these compounds (Bauernfeind and Chapman 1985; McClanahan and Founk 1983; Weber 1985).

L. pennellii (Corr.) D'Arcy, a wild relative of the cultivated tomato native to the western slopes of Peru, is a potential source of potato aphid resistance in tomato (Gentile and Stoner 1968; Quiros et al. 1977). Aphid resistance in *L. pennellii* has been attributed to the physical entrapment of the insects in the sticky exudate of the glandular trichomes (Gentile and Stoner 1968). *L. pennellii* possesses high densities of two morphologically distinct types of glandular trichomes, the type IV and VI (Lemke and Mutschler 1984). Type VI trichomes consist of a short 1–2 cell stalk with a membrane-enclosed tetralobulate glandular head (Luckwill 1943). The type IV trichomes of *L. pennellii* are slightly longer than the type

VI hairs and secrete a naked droplet of exudate at the tip. Although the cultivated tomato possesses high densities of the type VI hairs, type IV trichomes are completely absent.

However, entrapment is not *L. pennellii*'s only mechanism of insect resistance. Aphid feeding behavior on *L. pennellii* and its F_1 with tomato is characterized by an increase in the pre-probe time, and decreases in both the number of probes per unit time and the total time spent probing and feeding (Goffreda et al. 1988). Removal of the glandular exudate from *L. pennellii* and the F_1 with 95% ethanol increased aphid feeding, and the transfer of the glandular exudate to *L. esculentum* decreased aphid feeding as measured by these three feeding parameters (Goffreda et al. 1988). The differences in feeding behavior appear to be related to the presence of sugar esters in the glandular exudate of the type IV trichomes. Glucose esters from *L. pennellii* deter aphid settling when applied to a synthetic feeding membrane (Goffreda 1988).

The objectives of this study were to: (1) determine if *L. pennellii* is a strong, stable source of aphid resistance for the cultivated tomato, (2) elucidate the relationship between the type IV glandular hairs and aphid resistance in the field, (3) quantify each of the types of gene effects influencing aphid resistance in hybrids with tomato, and (4) estimate the heritability of this trait in backcross populations with tomato. An understanding about the genetic basis of aphid resistance in *L. pennellii* will facilitate the deployment of genes governing resistance in adapted tomato cultivars.

Materials and methods

Plant culture

Two aphid-susceptible tomato cultivars, New Yorker and VF Vendor, were hybridized to *L. pennellii* LA 716 (PI 246502) to produce F_1, F_2, and backcross progenies. Both tomato cultivars and this accession of *L. pennellii* have been selfed for numerous generations and were assumed to be homozygous.

Greenhouse-grown plants were scored for the presence and density of type IV trichomes on a leaflet adjacent to the terminal leaflet at the third node, 30 days after seeding. At this stage the seedlings were ca. 3.5 cm tall with the sixth leaf beginning to expand. Two determinations of the trichome density were made in a 6.6-mm^2 area, one on each side of the mid-vein, using a dissecting microscope at 60 ×. Since variances were proportional to treatment means in the non-segregating generations, variances were stabilized with a square root transformation of the trichome counts. The average of the square root of the number of type IV trichomes in each 6.6-mm^2 area was used in generation means analysis. Since the segregating generations contain greater genetic variation, the different generations were not equally represented. Within each replication, the non-segregating generations (P_1, P_2, and F_1) were represented by one plot, and the backcross and F_2 generations were represented by two and four plots, respectively. Each plot consisted of 14 plants. The experimental design was a split plot with two replications. Generations derived from crosses with New Yorker and VF Vendor were randomized in each replication.

Field-grown plants were evaluated for potato aphid resistance by 30-s counts of the number of potato aphids (red and green biotypes) on the plant, repeated at weekly intervals for a 4–5 week period during the season. This technique surveys a large portion of each plant for aphids, providing a relative estimate of aphid density. Since the standard deviation varied directly with the treatment mean in the non-segregating generations, a logarithmic transformation was used to stabilize the variance. Data were transformed by taking the log of the number of aphids plus one and averaging this value over the season.

In 1984, each plot consisted of 14 plants with an infestation plant (cv New Yorker) in the center of each row. Twenty apterous potato aphids (red biotype) were transferred to each infestation plant early in the season. Plots were sprayed with carbaryl (1/2–3/4 lb AI/acre) and chlorothalonil (0.5 lb AI/acre) to control aphid parasites, pathogens, and predators as proposed by Nanne and Radcliffe (1971). One plot of the non-segregating generations (P_1, P_2, and F_1) and two and four plots of the backcross and F_2 generations, respectively, were in each of four replications. The experimental design was a split plot with generations randomized within the four replications of each of the New Yorker and VF Vendor populations.

In 1985 and 1986, each plot consisted of ten plants. Aphids were not artificially introduced into the field in 1985 because there was an excellent natural infestation of potato aphids. In 1986, plants were inoculated with aphids by placing a potato aphid-infested tomato seedling (cv VFNT Cherry) on each plant in the field. In 1985 and 1986, there were two plots of the non-segregating generations (P_1, P_2, and F_1) and one plot of each BC_1F_2 family in each of two replications. Each year, either 19 or 20 BC_1F_2 families were evaluated in both the New Yorker and VF Vendor populations. Seed for the BC_1F_2 families were produced in the 1984 and 1985 field seasons by controlled self-pollinations of unopened flowers on random backcross to *L. esculentum* plants (cultivars New Yorker and VF Vendor). Plots were sprayed with carbaryl and chlorothalonil, as previously described in this section.

Statistical and genetic analysis

An analysis of variance of potato aphid resistance (as measured by the transformed insect counts in the 1984–1986 growing seasons) was performed on the non-segregating generations (P_1, P_2, and F_1) derived from crosses with New Yorker and VF Vendor. Data were analyzed within each year since the genotype × year interaction was highly significant ($P<0.0001$). The mean transformed aphid count from each plot was analyzed as a single experimental unit. Statistical significance between means was determined by a Bonferroni *t*-test ($P<0.05$) (Miller 1981).

Weighted least-squares were used to estimate genetic parameters in joint scaling tests with weights that were equal to the reciprocals of the standard errors of the generation means, as proposed by Mather and Jinks (1971). The computational procedures used were those described by Rowe and Alexander (1980), using expected coefficients of the gene effects presented by Hayman (1958) (Table 1). This model defines the average effect of a gene substitution in terms of the linear regression of the genotypic value on number of alleles at a given locus as proposed by Fisher (1941), and it assumes that all the favorable alleles are in *cis* and contributed by *L. pennellii*. Transformed trichome density from the 1984 greenhouse study and aphid count data from the 1984 field study were subjected to analysis by generation means using the full 6-parameter model. The model was reduced to a simple additive-dominance model by sequential removal of gene effects from the model whenever possible. The validity of the reduced 3-parameter and 5-param-

Table 1. Expected coefficients of gene effects for the six generation means

Generation	Gene effects[a]					
	m	[d]	[h]	[i]	[j]	[l]
P_1	1	1	−0.5	1	−1	0.25
P_2	1	−1	−0.5	1	1	0.25
F_1	1	0	0.5	0	0	0.25
F_2	1	0	0	0	0	0
BC_1P_1	1	0.5	0	0.25	0	0
BC_1P_2	1	−0.5	0	0.25	0	0

[a] m – mean, [d] – additive, [h] – dominance, [i] – additive × additive, [j] – additive × dominance, and [l] – dominance × dominance effects

Table 2. Mean potato aphid (*Macrosiphum euphorbiae*) counts per 30 s on plants of *L. pennellii* (LA 716), *L. esculentum* (cultivars 1. New Yorker and 2. VF Vendor), and their F_1 hybrids

Cross/ Generation		Pedigree	Year		
			1984	1985	1986
1	P_1	*L. pennellii* (LA 716)	2.6a	20.7a	3.7a
	P_2	*L. esculentum* cv New Yorker	33.5b	78.2c	67.8c
	F_1	$(P_2 \times P_1)$	3.2a	28.2b	40.1b
2	P_1	*L. pennellii* (LA 716)	2.0a	7.4a	1.8a
	P_3	*L. esculentum* cv VF Vendor	33.8b	91.2c	56.0c
	F_1	$(P_3 \times P_1)$	2.2a	24.9b	28.5b

a, b, c – Means followed by a different letter within each cross and year differ significantly by a Bonferroni *t*-test ($P < 0.05$). Data were analyzed by using an analysis of variance on the transformed data (log+1). Table presents original treatment means

eter models were tested with a Chi-square goodness-of-fit test with 3 and 1 *df*, respectively.

Standard unit heritability estimates were obtained by parent-offspring regression as proposed by Frey and Horner (1957). Parent BC_1F_1 plants were standardized by subtracting the mean of the transformed aphid counts and dividing by the standard deviation. Offspring were grown the following year and their plot means were averaged, standardized, and regressed on their parent standard scores. The regression coefficient (β_1) overestimates heritability (h^2) because of prior inbreeding in the base population (Smith and Kinman 1965). Heritability estimates were calculated by dividing the regression coefficient (β_1) by two times the coefficient of parentage (r_{xy}). Since the coefficient of parentage between BC_1F_1 parents and their BC_1F_2 offspring is equal to 3/4, heritability estimates were calculated by multiplying the regression coefficient by 2/3. This estimate of heritability is biased upwards by both dominance and epistatic variation.

Results and discussion

Potato aphid resistance in L. esculentum, L. pennellii and their F_1 hybrids

L. pennellii (LA 716) appears to possess both a stable and high level of resistance to the potato aphid. Aphid populations were sparse on *L. pennellii* even in 1985 when there was an extremely high infestation of aphids (Table 2). Although alate aphids would settle on *L. pennellii* plants, the aphids were unable to establish thriving colonies as they did on both cultivars of *L. esculentum*. Resistance in the F_1 varied from almost complete immunity to aphids in the 1984 growing season, to moderate levels of aphid resistance in the 1985 and 1986 season. The level of aphid resistance in *L. pennellii* and the F_1 differed significantly from the *L. esculentum* controls in all 3 years (Table 2). Aphid resistance in *L. pennellii* and the F_1 differed significantly in both 1985 and 1986, but not in 1984. Data from the 1984 and 1985 seasons indi-

Table 3. Segregation ratios in *L. esculentum* × *L. pennellii* hybrids for the presence of type IV glandular hairs

Cross/Generation		Pedigree	Type IV trichomes				χ^2	*P*
			Observed		Expected			
			Present	Absent	Present	Absent		
1	P_1	*L. pennellii* (LA 716)	28	0	28	0		
	P_2	*L. esculentum* cv New Yorker	0	28	0	28		
	F_1	$(P_2 \times P_1)$	28	0	28	0		
	F_2	$(P_2 \times P_1)^2$	107	5	105	7	0.609[a]	$0.5 > P > 0.25$
	BC_1	$(P_2 \times P_1) \times P_1$	56	0	56	0		
	BC_1	$(P_2 \times P_1) \times P_2$	36	20	42	14	3.428[b]	$0.1 > P > 0.05$
2	P_1	*L. pennellii* (LA 716)	28	0	28	0		
	P_3	*L. esculentum* cv VF Vendor	0	28	0	28		
	F_1	$(P_3 \times P_1)$	28	0	28	0		
	F_2	$(P_3 \times P_1)^2$	107	5	105	7	0.609[a]	$0.5 > P > 0.25$
	BC_1	$(P_3 \times P_1) \times P_1$	56	0	56	0		
	BC_1	$(P_3 \times P_1) \times P_3$	37	17	40.5	13.5	1.210[b]	$0.5 > P > 0.25$

[a] Tested against a 15:1 ratio
[b] Tested against a 3:1 ratio

cate that aphid resistance in the F_1 hybrids exhibits at least partial dominance and that the level of dominance observed varies with aphid pressure. The 1986 data does not support dominance for resistance.

Inheritance of type IV trichomes in L. esculentum × L. pennellii hybrids

Data from the segregating generations indicate that the presence of the type IV trichomes on the seedlings is controlled by two dominant, unlinked genes, with either gene conferring the type IV trichome in the hybrids. Data from the F_2 and backcross to *L. esculentum* generations in crosses with either tomato parent fit a 15:1 and 3:1 ratio, respectively (Table 3). All F_1 and backcross to *L. pennellii* plants examined possessed type IV trichomes. These data support the conclusions of Lemke and Mutschler (1984) that the presence of the type IV trichomes is simply inherited, controlled by duplicate gene epistasis.

The mean type IV trichome densities on F_1 and F_2 plants with either tomato cultivars were intermediate between tomato and *L. pennellii* (Table 4). Backcrossing to *L. pennellii* increased the mean type IV trichome density, whereas backcrossing to *L. esculentum* lowered trichome densities. In the F_2 populations derived from crosses with New Yorker and VF Vendor, 22% and 21% of the plants, respectively, had type IV trichome densities within the range of densities obtained on *L. pennellii*. In the backcross to *L. pennellii*, 50% and 57% of the plants in the New Yorker and VF Vendor populations had trichome densities within *L. pennellii*'s range. These data suggest that the density of type IV trichomes is simply inherited, controlled by only a few major genes. Variation in the mean density of type IV trichomes in the populations derived from crosses with New Yorker and VF Vendor was adequately explained by the 3-parameter, additive-dominance model in generation means analysis (Table 5).

Inheritance of aphid resistance in L. esculentum × L. pennellii hybrids

The F_1 hybrids with both tomato cultivars and their backcross generations to *L. pennellii* exhibited levels of aphid resistance which were similar to that of *L. pennellii* (Table 4). However, the mean level of aphid resistance in the F_2 and backcrosses to *L. esculentum* generations decreased dramatically, suggesting that epistatic interactions between loci could be important in the genetic mechanism of aphid resistance. Few F_2 and backcross to *L. esculentum* plants possessed as high a level of aphid resistance as the F_1 generation, indicating that aphid resistance exhibits complex inheritance.

The additive-dominance model could not adequately explain the variation in the mean level of aphid resistance, suggesting that epistatic gene effects may strongly influence resistance ($\chi^2 = 238.7$ and 264.9, $df = 3$, New Yorker and VF Vendor populations, respectively; data not shown). In the populations derived from crosses with New Yorker, the generation means were adequately described by a 5-parameter model which also included additive × additive and additive × dominance digenic epistatic effects (Table 5). No reduced model could adequately explain the variation in the mean level of aphid resistance in the VF Vendor-derived generations.

Table 4. Generation means of type IV trichome densities and potato aphid (*Macrosiphum euphorbiae*) counts on *L. esculentum* × *L. pennellii*

Cross/ Generation		Pedigree	Trichome density (no./6.6 mm²)		$\sqrt{\text{density}}$ ($\sqrt{\text{no./6.6 mm}^2}$)		Av. aphid count (no./30 s)		Av. transformed aphid count (Log[no. + 1]/30 s)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	P_1	*L. pennellii* (LA 716)	172	27	13.1	1.0	2.6	2.9	0.32	0.24
	P_2	*L. esculentum* cv New Yorker	0	0	0.0	0.0	33.5	19.5	1.21	0.33
	F_1	$(P_2 \times P_1)$	100	39	9.8	1.9	3.2	4.7	0.31	0.29
	F_2	$(P_2 \times P_1)^2$	85	47	8.7	2.9	28.5	24.5	1.12	0.47
	BC_1	$(P_2 \times P_1) \times P_1$	128	46	11.1	1.9	4.5	8.4	0.40	0.35
	BC_1	$(P_2 \times P_1) \times P_2$	34	37	4.7	3.5	31.7	24.1	1.14	0.38
2	P_1	*L. pennellii* (LA 716)	194	29	13.9	1.0	2.0	1.9	0.29	0.20
	P_3	*L. esculentum* cv VF Vendor	0	0	0.0	0.0	33.8	23.8	1.15	0.41
	F_1	$(P_3 \times P_1)$	88	15	9.3	0.8	2.2	4.6	0.19	0.26
	F_2	$(P_3 \times P_1)^2$	77	50	8.2	3.2	25.6	23.5	1.04	0.49
	BC_1	$(P_3 \times P_1) \times P_1$	132	44	11.3	1.9	6.4	10.6	0.47	0.39
	BC_1	$(P_3 \times P_1) \times P_3$	29	30	4.6	2.9	45.7	34.6	1.29	0.48

Relationship between type IV trichomes and aphid resistance

Previous research has demonstrated that compounds secreted from the type IV trichomes of *L. pennellii* and the F_1 strongly deter potato aphid settling and feeding (Goffreda 1988; Goffreda et al. 1988). However, we have not detected a significant relationship between type IV trichome densities on seedlings and field resistance to aphids in segregating F_2 and backcross generations (data not shown). It is possible that seedling determinations of trichome densities may not be highly correlated with trichome densities of adult, field-grown plants. Although a simple additive-dominance model could explain the variation in type IV trichome densities on 30-day-old seedlings in this study, Lemke and Mutschler (1984) found that epistatic gene effects were necessary components for the model to explain the variation in generation means of 50-day-old seedlings. The principal difference between the two studies was the age of the plants at the time of trichome determination; perhaps genes involved in differential rates of leaf expansion as the plants mature are responsible for the significant epistatic gene effects detected in the study by Lemke and Mutschler (1984).

Another possible explanation for the lack of correlation between trichome density and aphid resistance is that the trichomes of many hybrids may lack or have substantially reduced quantities of the compounds responsible for the insect resistance. Resistance in *Nicotiana* spp. to the green peach aphid, *Myzus persicae* Sulzer, increases as the plant matures, which parallels an increase in the amount of trichome exudate (Abernathy and Thurston 1969). In the present study, droplet size of the type IV trichomes in the F_2 and backcross to *L. esculentum* generations was very variable between plants and also appeared to be strongly influenced by the age of the plant and environmental conditions. The large magnitude of epistatic effects on aphid resistance, as indicated by the generation means analysis, could result from the interaction between genes governing the presence of the type IV trichomes and those responsible for the production of the biologically active compounds.

Estimation of heritability by parent-offspring regression

Potato aphid infestations were not uniform in 1984, resulting in poor evaluation of the level of aphid resistance of the parental BC_1F_1 base population grown in 1984. Consequently, the regression coefficients and standard unit heritability estimates calculated from the 1984 parental data were lower than those in 1985, when aphid infestations were high (Figs. 1 and 2). In the 1985–1986 growing seasons, potato aphid resistance exhibited a moderate level of heritability ($29.8\% \pm 14.1\%$ and $47.1\% \pm 11.5\%$ for the New Yorker and VF Vendor backcross populations, respectively). Heritability estimates were generally low in 1984–1985 growing seasons ($16.1\% \pm 15.7\%$ and $21.9\% \pm 14.8\%$ for the New Yorker and VF Vendor backcross populations, respectively). Consequently, single plant selections based on the repeated aphid counts will only be effective in years of uniformly high aphid infestations.

Table 5. Estimates of gene effects for type IV trichome density and aphid resistance in *L. esculentum* × *L. pennellii* hybrids

Cross[a]	Component[b]	Trichome density		Aphid resistance	
		6-parameter	Reduced model (±SE)	6-parameter	Reduced model (±SE)
1	m	8.74	8.22±0.035	1.12	1.09±0.003
	[d]	6.48	6.47±0.065	−0.74	−0.74±0.008
	[h]	−0.06	3.26±0.253	−1.85	−1.60±0.035
	[i]	−3.33	–	−1.40	−1.14±0.021
	[j]	−0.07	–	−0.30	−0.30±0.011
	[l]	4.40	–	0.47	–
χ^2 (df=1 or 3)			5.47[NS]		3.48[NS]
2	m	8.16	8.11±0.002	1.04	1.10±0.012
	[d]	6.74	6.91±0.004	−0.82	−0.78±0.046
	[h]	1.62	2.38±0.012	−1.17	−1.75±0.122
	[i]	−0.77	–	−0.65	−1.24±0.086
	[j]	−0.20	–	−0.39	−0.34±0.058
	[l]	1.44	–	−1.06	–
χ^2 (df=1 or 3)			0.98[NS]		13.72**

[NS, **] Not significant and significant at the 1% level, respectively

[a] Cross 1 – New Yorker × LA 716 hybrids; cross 2 – VF Vendor × LA 716 hybrids

[b] m – mean, [d] – additive, [h] – dominance, [i] – additive × additive, [j] – additive × dominance, and [l] – dominance × dominance effects

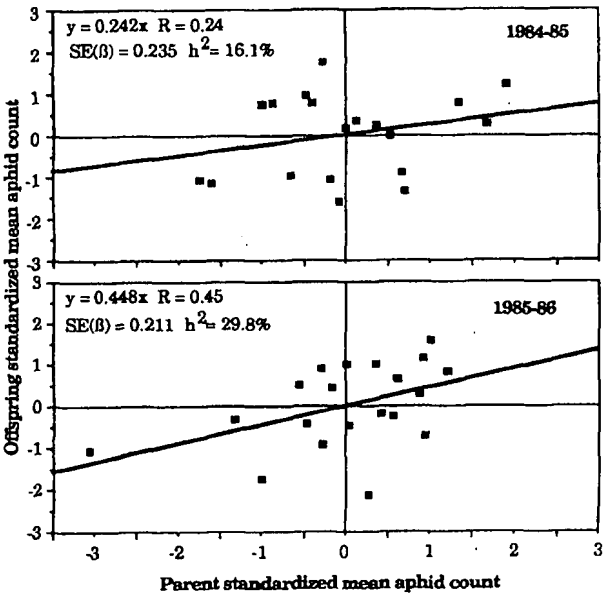

Fig. 1. Regression of standardized aphid count data from BC_1F_2 families on BC_1F_1 parents derived from backcrosses to *L. esculentum* cv New Yorker

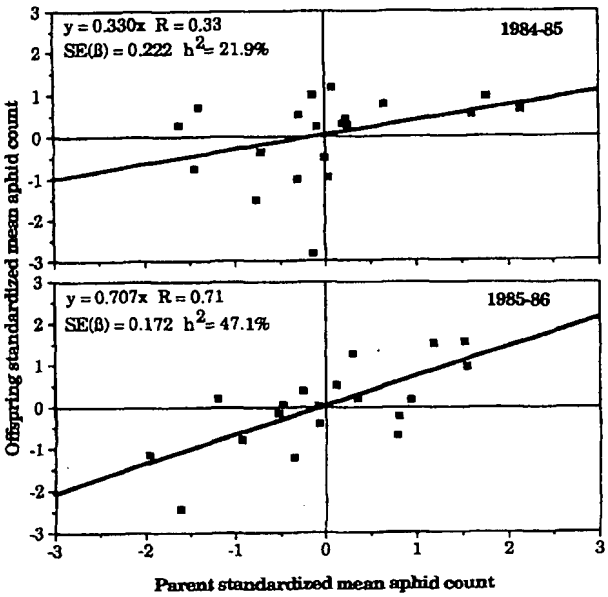

Fig. 2. Regression of standardized aphid count data from BC_1F_2 families on BC_1F_1 parents derived from backcrosses to *L. esculentum* cv VF Vendor

These data suggest that selection for potato aphid resistance will be more efficient if delayed until later generations because of the large epistatic effects and the low heritability of this trait in seasons with reduced aphid infestations. However, trichome characteristics can be selected in early generations since they appear to be simply inherited. Selection for type IV trichome density alone may not be effective, because the trichomes on different plants in segregating populations may vary in their ability to produce biologically active compounds. Sugar esters present in the type IV exudate strongly deter aphid settling and feeding (Goffreda 1988; Goffreda et al. 1988). We have developed a technique to quantify the level of sugar ester production on the leaf surface and are determining whether this technique could be an effective selection tool in the development of aphid resistant tomato varieties.

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